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Biometry of Puffers and Their Parasites.

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INTRODUCTION.

In connection with other investigations of the New York Aquarium, studies were made of fishes and fish parasites indigenous to Sandy Hook Bay, N. J. The fishes of this area have been thoroughly studied by Breder (1923-1939) and his collaborators. Among other species, the northern puffer or blowfish, *Spheroides maculatus* (Bloch & Schneider), is found there in large numbers from June to September and is collected in these months for exhibition purpose.

A routine check for parasites is made on all fishes that eventually will become part of the Aquarium's exhibition. In the course of these investigations it was evident that puffers were the foci for infestations that were responsible for mortalities of other and more valuable species of fishes. For this reason, it was decided that a survey of their parasitic fauna would yield additional information in this regard and that perhaps such a study would suggest methods for their control.

SAMPLINGS.

A random selection of puffers was made when the specimens were brought into the Aquarium from the pound nets in Sandy Hook Bay. A total of 294 fish was taken, varying in size from 2 to 24 cm. In Table I the number of individuals collected in the months from June to October, inclusive, for the years 1938, 1939 and 1940, are shown. The largest number of puffers was caught in September for the three years. Table II gives the numbers of each sex collected during the same period. 49% of these were males; 44% were females; 7% were immature. The males varied in size from 10-20 cm.; females from 11-24; immature puffers measured up to 12 cm. In this collection, fish 21 cm. or more were females. This distribution is shown in Table III and agrees with the data given by Welsh & Breder (1922) for puffers caught off Atlantic City, N. J. The only difference noted is the slight extension for the mature forms in both the upper and lower size limits, the result of a larger sample.

ANALYSIS OF THE INTESTINAL CONTENTS OF PUFFERS.

The intestinal contents of puffers were examined by Linton (1901, 1905) from Woods Hole, Mass., and Beaufort, N. C., and by Welsh & Breder (1922) from Atlantic City. These investigators found that the puffer's diet consisted of minute crustaceans, crabs, shrimps, bivalve and univalve mollusks, annelids and sea-weeds. In addition to such items, fish was also part of the diet of Sandy Hook puffers.

It was Ward (1908) who stated that "The parasitic fauna of any animal is primarily a function of its habitat" and, one may add, of its eating habits. A knowledge of the food cycle is very important, often revealing the source of internal parasites. Thus, for the five trematodes reported in this paper (see Table IV), the infective stages are to be found in mollusks, crustaceans and fishes. Martin (1939) showed that in the case of *Stephanostomum tenue*, one of the intestinal flukes of the puffer, the cercariae develop in the snail, *Nassa obsoleta*, and the metacercariae are found in the spearing or common silverside, ***Menidia menidia notata***. The spearing is a small fish occurring in great abundance along our coast and is usually preyed upon by larger fish. It is not surprising, therefore, to find this species of fluke living in a large variety of definitive hosts.

In the life-histories of *Lecithaster* and *Podocotyle* (see Hunnien & Cable, 1941 a and b), the cercarial stages appear in certain snails and the metacercariae or infective forms occur unencysted in copepods and amphipods. Since these minute crustaceans are part of the diet of many marine fishes, it is to be expected that no host-specificity is shown by these trematodes. The cycles of *Bianium* and *Lintonium* are still unknown but they are almost certain to follow one of the patterns indicated above.

Parasites of the Puffer.

A. External Parasites.

Insofar as could be determined, about 18 species of parasites have been recorded from the puffer. Thirteen of these were found in Sandy Hook Bay fish and are listed in Table IV.

The small opercular opening, characteristic of puffers, encourages the concentration of ectoparasites in the gill chambers. In regard to pathogenicity, ectoparasites of fishes are the most important ones to consider for invariably they cause death of the host if sufficient numbers are present.

Table I.

Number of Puffers Collected, According to Dates.

Month	1938	1939	1940	Total
June	7	6	12	25
July	—	15	69	84
August	20	20	19	59
September	39	54	30	123
October	1	2	—	3
Total	67	97	130	294

The dinoflagellate, *Oodinium ocellatum* Brown, is recorded from only two fish in this report but in previous years (1935-1937) it was the cause of mortality of various marine fishes (tropical and temperate) kept in captivity. The life-history of *Oodinium* was demonstrated by Nigrelli (1936) and it was shown that the epidemic in the Aquarium was centered in puffers and spiny boxfish brought in from Sandy Hook Bay.

The two species of *Trichodina* were described recently by Padnos & Nigrelli (1942). Heavy infestations of these ciliates will cause death. Nigrelli (1940) showed that puffers were the source for *Trichodina* found on other fishes in the Aquarium. That these peritrich ciliates are not limited to Sandy Hook fish is indicated by the fact that in 1939 they were also present on the gills and skin of 27 out of 32 puffers examined from Orient Point, L. I. The protozoa reported by Linton (1940) as *Cyclochaeta domerguei* Moroff (p. 23) may be *Trichodina*. If this is true, it appears that the puffers are infected with the organism throughout its known range.

Tables V and VI show the relationship of the intensity of *Trichodina* infection to sex of the host and to the dates the puffers were taken. Fish showing an exceptionally light infection may be considered negative for it is suspected that some of the gills probably were contaminated at the time of the examinations. As will be seen later, the host-parasite relation shown here is opposite to that demonstrated for the metazoan parasites of the puffers. There is a tendency towards an increase infection in males. The intensity of the infection in all fish decreases towards the latter part of the summer. The effect of temperature has not been definitely decided. Our records show that the temperature in the bay off the Battery reaches a maximum in August and begins to drop in the latter part of the month. Table VI shows that there may be a correlation, and this is to be expected, between the temperature and growth of the ciliates. The Table also indicates that the optimum is around 70° F., for it is at this temperature that the largest number of puffers were infected. This interpretation is further corroborated by the fact that most of the reproductive stages (conjugation) of this organism were obtained from the gills of puffers caught in July and September.

A single myxosporidian cyst was found on the gills. The spores are bivalve and more or less oval in shape, indicating that this form should be allocated to the family Myxosomatidae. No specific identification was made because of insufficient material. However, since the mode of transmission is direct, such organisms must be considered as potential parasites of real importance for under epidemic conditions they could cause considerable damage to tissues (Nigrelli & Smith, 1938) and eventually result in death.

The copepod, *Pseudochondracanthus diceraus* Wilson (see Wilson, 1932), is another important gill parasite of puffers. As many as 140 specimens were found attached to the gills of a single fish. The method of transmission is simple and direct. The fertile

Table II.

Number of Puffers Collected, According to Sex.

	1938			1939			1940			Total			Grand
Month	M	F	I	M	F	I	M	F	I	M	F	I	Total
June	2	5	—	2	4	—	4	8	—	8	17	—	25
July	—	—	—	7	8	—	40	29	—	47	37	—	84
August	2	4	14	10	7	3	13	6	—	25	17	17	59
September	18	21	—	28	24	2	18	12	—	64	57	2	123
October	—	—	1	1	1	—	—	—	—	1	1	1	3
Total	22	30	15	48	44	5	75	55	—	145	129	20	294

TABLE III.

Collection of Puffers: Relation of Size to Sex.

Size in cm.	M	F	I	Total
2	—	—	1	1
3	—	—	2	2
4	—	—	2	2
5	—	—	1	1
6	—	—	2	2
7	—	—	4	4
8	—	—	—	—
9	—	—	2	2
10	4	—	—	4
11	10	4	2	16
12	7	9	4	20
13	13	5	—	18
14	18	4	—	22
15	21	7	—	28
16	25	7	—	32
17	20	14	—	34
18	10	18	—	28
19	14	18	—	32
20	3	16	—	19
21	—	10	—	10
22	—	8	—	8
23	—	8	—	8
24	—	1	—	1
Total	145	129	20	294

eggs hatch into infective stages (copepodid) which attach themselves immediately to the gills. It is obvious that if sufficient numbers are present on the delicate gill filaments interference with the normal respiratory function will occur.

B. Internal Parasites.

1. Trematodes.

All the trematodes recovered from the puffers are digenetic. Of the five species found in the Sandy Hook puffers, *Lintonium vibex* (Linton) (Stunkard & Nigrelli, 1930) and *Bianium plicitum* (Linton) (Stunkard, 1931) are the only ones found in appreciable numbers and with any degree of constancy. They occur in the digestive tract and were present, respectively, in 66% and 67% of the fish examined.

Lintonium vibex appears to be species-specific. It has not been recorded from any other fish occurring naturally in the North Atlantic. Linton (1940) reported an infestation in a queen triggerfish, *Balistes vetula*, which had straggled northward to the Woods Hole region during the summer. The triggerfish probably became infected when it migrated into the northern waters. Further, this species, examined for internal parasites from its natural habitat, has not been reported as a host for *Lintonium*. Close relatives of the puffer, such as the rabbit fish or smooth puffer, *Lagocephalus laevigatus*, and the common spiny boxfish, *Chilomycterus schoepfii*, taken at Sandy Hook Bay at the same time that puffers were seined, were free of this trematode. Both of these fish have feeding habits similar to the common puffer.

Bianium plicitum is usually present in puffers in large numbers. The parasite was originally described as an unidentified species by Linton in 1898 from two specimens found in the smooth puffer and the tom cod, *Microgadus codus*, and again in 1929 as *Psilostomum plicitum* from the intestine of the herring gull, *Larus argentatus*. It was shown by Stunkard (1931) that the two forms were identical with a species he had described in 1930 as *Bianium concavum*, a common intestinal parasite of the puffer and for this reason was designated *B. plicitum*. This important discovery shows definitely, what often has been suspected, that parasites which are normally found in one host may persist for a time in its predators. The presence of *Bianium* in the smooth puffer and the tom cod may be accounted for in this manner. From the record so far presented, it seems that *Bianium plicitum* also is a specific puffer parasite.

Podocotyle olssoni Odhner, *Lecithaster confusus* Odhner and *Stephanostomum tenue* (Linton) have been described from a variety of North Atlantic fishes. Their life-histories were discussed above, and although together they were found in 8% of the puffers, individually there were never enough present at one time to make them significant.

Cymbephallus vitellosus (Linton) is another intestinal fluke reported for puffers from Woods Hole region. It is closely related to *Podocotyle* and like this form is found in a large number of fishes in this locality.

2. Cestodes.

The striking feature of the present studies is the dearth of cestodes. A single, unidentified scolex was found. Linton (1924) reported more than 24 encysted and free scoleces from about 20 puffers examined from Woods Hole. From this material he was able to identify *Rhynchobothrium bulbifer*, *R. tumidulum* and *Tetrarhynchus bisulcatus*. These identifications are incomplete because, as pointed out by Nigrelli (1938), the names *Tetrarhynchus* and *Rhynchobothrium* have no generic status. They are group names. *Tetrarhynchus bisulcatus* Linton has since been redesignated *Nybelinia bisulcata* (Linton) Dollfus. Larval cestodes occur in numerous teleost hosts and although their life-cycles are not completely understood, it is known that they become sexually mature in the spiral valve regions of the intestines of elasmobranchs.

3. Nematodes.

Thirty-six per cent of the puffers were infested with nematodes belonging to the genera *Contracaecum* and *Porrocaecum*. No

TABLE IV.

List of the Parasites Collected from Puffers of Sandy Hook Bay, N. J.

Parasite	Site of Infestation	M	F	I	Total	Ratio of Infect. %
1. *Oodinium ocellatum* (Dinoflagellate)	Gills and Skin	—	2	—	2	.7
2. *Trichodina spheroidesi*	Gills and Skin	126	113	6	245	83.0
3. *Trichodina halli* (Ciliate)	Gills and Skin					
4. Myxosporidian (sp. ?) (Cnidosporidia)	Gills	—	1	—	1	.3
5. *Lintonium vibex* (Trematode)	Pharynx	101	82	—	183	66.0
6. *Bianium plicitum* (Trematode)	Intestine	98	98	—	196	67.0
7. *Podocotyle olssoni* (Trematode)	Intestine	9	5	—	14	5.0
8. *Lecithaster confusus* (Trematode)	Intestine	3	1	—	4	1.4
9. *Stephanostomum tenue* (Trematode)	Intestine	1	4	—	5	2.0
10. Tapeworm scolex (sp. ?)	Intestine	1	—	—	1	.3
11. *Contracaecum* sp. (Nematode)	Body Cavity	29	30	—	59	20.0
12. *Porrocaecum* sp. (Nematode)	Intestine	24	23	—	47	16.0
13. *Pseudochondracanthus diceraus* (Copepod)	Gills	106	98	—	207	70.0

specific identification was made because they are difficult to distinguish from any one of a number of species reported from marine fishes. Both forms are larval in character. *Contracaecum* is invariably found coiled in delicate cysts in the retroperitoneal regions of the liver, intestine and other organs. Sexually mature stages are known to occur in piscivorus birds and mammals. *Porrocaecum* was found free in the intestine of puffers. These probably become mature in elasmobranchs, marine turtles or seals.

The acanthocephalan, *Echinorhynchus acus*, reported by Linton (1901) from the pharynx of a single puffer, was not present in our material nor has it been recorded from puffers since that time. This must be an accidental infestation.

EFFECTS OF INTERNAL PARASITES ON THE HOST.

There was no evidence to indicate any real pathological effects in instances of severe infestations with the internal parasites just described. The presence of large numbers of flukes must without doubt elicit some physiological response not often obvious in the course of autopsy. In certain instances it has been noted that intestinal flukes in fishes are capable of causing an enteritis (Nigrelli, 1940), excess production of mucus and a superficial necrosis of the mucus coat.

It is interesting to point out that in infestations with *Bianium*, all of the individuals appeared to be at about the same stage of development (as indicated by size), suggesting that puffers obtain a single dose. On the other hand, individuals of *Lintonium* were found varying in size from minute, hardly perceptible forms to large, sexually mature specimens. This shows that puffers become infested continuously with *Lintonium* and that an initial infestation does not confer any resistance to the host. Whether or not the one infestation with *Bianium* is sufficient for puffers to develop an immunity against a further attack of this parasite is not known. That the infestation is less stable than that of *Lintonium* is demonstrated by the observation that puffers subjected to a period of captivity lose most or all of the *Bianium* while *Lintonium* may persist indefinitely.

OCCURRENCE OF PARASITES OF PUFFERS ACCORDING TO DATES OF CAPTURE.

Table VII summarizes the data regarding the number of parasites collected from the 294 puffers examined. As may be seen, the intensity of infestation reaches a peak in July and sharply drops off in August. For the first two months, 109 fish were examined in the three years from which 5,022 specimens of the three species were counted. In the last three months 185 fish examined yielded only 1,328 parasites. Although 25%

TABLE V.

Intensity of *Trichodina* Infection in Relation to Sex.

Intensity of Infection*	Male	Female	Immature	Total	%
Exceptionally Heavy	7	8		15	
Very Heavy	5	10	1	16	
Heavy	18	18		36	
					23.00
Medium	9	6		15	
Light	29	29	1	59	
Very Light	19	20	3	42	
					39.00
Exceptionally Light	39	22	1	62	
Negative	19	16	14	49	
					38.00
Total	145	129	20	294	
					100.00

* *Exceptionally Heavy*, too many in the field to count; *Very Heavy*, about 100 ciliates in each field; *Heavy*, about 50 in each field; *Medium*, about 25 in each field; *Light*, about 12 in each field; *Very Light*, 1 or 2 in each field; *Exceptionally Light*, 1 or 2 in an occasional field. Note: Some of the last mentioned may be contamination.

more fish were examined during the latter period, the parasite fauna was 60% less. It should be indicated here that during June and July all females examined were gravid while most fish caught in August and September had spawned. The great decrease in the numbers of parasites, during the latter period, as shown below, may be attributed partly to the spawning activities of the fish.

OCCURRENCE OF PARASITES IN RELATION TO SIZE AND SEX OF PUFFERS.

Table VIII shows the total number of parasites, excluding the protozoa, in relation to host size. It will be seen that the number of parasites increases rapidly with the size and age of the fish. Conversely, puffers up to 10 cm. in length were particularly free of parasites. A total of 6,520 parasites were counted. 66% of these were taken from female puffers, although there were fewer females than males in the total catch. This does not necessarily mean that females are more susceptible because of their larger size. Table IX shows the relation of the infestation to sex, according to dates the fish were caught. In this series a total count of the three major parasites was made. The figures show that the female puffers are susceptible to heavier infestations. This is partly corroborated by the data given in Table X showing fish infected simultaneously with the three major parasites. It will be noted that in puffers measuring from 16-19 cm., inclusive, the number of males and females collected was about equal (33 and 32 respectively), yet the females had at least 50% more parasites than the males. The greater susceptibility of females to parasitic infestation may be attributed in part to their more intensive feeding preparatory to spawning. This Table also shows that *Bianium* is present in larger numbers.

PUFFERS SIMULTANEOUSLY INFESTED WITH THE THREE MAJOR PARASITES (EXCLUDING PROTOZOA).

An analysis was made to determine whether or not a heavy infestation of one kind of parasite would confer on puffers a certain amount of resistance to other types. Table X shows that this does not happen. About 45% of the fish were simultaneously infested with *Pseudochondracanthus*, *Lintonium* and *Bianium*. Furthermore, this 45% was infested with about 84% of these parasites.

TABLE VI.

Intensity of *Trichodina* Infection in Relation to Dates.

Month	Av. Temp.*	Heavy to Except. Heavy Infect.	Medium to Light Infect.	Except. Light to Neg. Infect.	Total
June	63.0F.	10	10	5	25
July	68.0	23	33	28	84
August	72.0	8	20	31	59
September	70.0	25	53	45	123
October	66.0	1	—	2	3
Total		67	116	111	294

* Average temperature in Bay off Battery Park for years 1938, 1939, 1940.

TABLE VII.

Number of Parasites Collected (Three Species Only), According to Dates.

Month	Copepod	*Lintonium*	*Bianium*	Total
June	219	129	1108	1456
July	917	433	2216	3566
August	195	192	263	650
September	161	248	268	677
October	—	1	—	1
Total	1492	1003	3855	6350

TABLE VIII.

Size of Host and the Total Number of Parasites Collected from Puffers (Excluding Protozoa).

Size in cm.*	Male	Female	Immature	Total
2	—	—	—	—
3	—	—	—	—
4	—	—	—	—
5	—	—	—	—
6	—	—	—	—
7	—	—	—	—
8	—	—	—	—
9	—	—	2	2
10	62	1	—	63
11	151	1	1	153
12	111	55	4	170
13	132	198	—	330
14	175	65	—	240
15	199	92	—	291
16	265	38	—	303
17	327	190	—	517
18	230	698	—	928
19	493	784	—	1277
20	86	573	—	659
21	—	713	—	713
22	—	442	—	442
23	—	331	—	331
24	—	101	—	101
Total	2231	4282	7	6520

*Standard length

FREQUENCY DISTRIBUTION OF PARASITES OF PUFFERS, EXCLUDING PROTOZOA, (CONSIDERED IN GROUPS OF 25).

Table XI shows the frequency with which certain numbers of parasites occur in puffers when considered in groups of 25. As may be seen 11% of the puffers were free of infection. 63% of the fish had from 1-25 parasites. Fish with more than 100 parasites were all females. However, they comprise only about 3% of the catch.

SUMMARY AND CONCLUSIONS.

1. The parasitic fauna of puffers from Sandy Hook Bay, N. J., were studied.
2. Two hundred and ninety-four fish were collected in the months from June to October inclusive, for the years 1938, 1939, 1940. The largest number of fish were caught in September for the three years.
3. The size range of the host extended from 2-24 cm. Fish 9 cm. or less were immature, although they may reach a length of 12 cm.; males measured from 10-20 cm.; females from 11-24 cm. Puffers 21 cm. or more in length were all females.
4. Food eaten by puffers consists of copepods, amphipods, crabs, shrimps, bivalve and univalve mollusks, annelids, fish and sea-weeds. The life-cycle of internal parasites, particularly the flukes, involves any one of these as intermediate host.
5. Thirteen kinds of parasites were found: 4 species of protozoa, 5 species of digenetic trematodes, 1 tapeworm, 2 nematode species and 1 copepod. The protozoans and the copepods are gill parasites, heavy concentrations of which will cause death of puffers and other marine fishes.
6. In reference to numbers, the important metazoan parasites of the puffer are the flukes, *Lintonium vibex* and *Bian-*

ium plicitum, and the copepod, *Pseudochondracanthus diceraus*.

7. Both *Lintonium vibex* and *Bianium plicitum* are specific to puffers of the North Atlantic coast of the United States.
8. Fishes related to the puffers (rabbit fish and queen triggerfish) migrating into this area may become infested with these flukes. However, other relatives of puffers, such as the spiny boxfish, living in the same area and apparently with the same feeding habits, seem to be resistant to *Lintonium* and *Bianium*.
9. From observations, it appears that puffers are infested continuously with *Lintonium* while in the case of *Bianium* only one infestation occurs at a time.
10. In captivity, the puffers lose their *Bianium* after a short period while *Lintonium* may persist indefinitely.
11. Other species of flukes recovered from Sandy Hook Bay puffers have also been reported from a large variety of fishes of the North Atlantic. In some cases, these parasites may be accidental infestations.
12. Although a number of species of cestodes have been described from puffers, only a single unidentified scolex was recovered from Sandy Hook Bay fish.
13. The two species of nematodes found were larval in character and presumably sexually mature forms come up in piscivorous hosts.

TABLE IX.

Number of Parasites Collected (Three Species Only), According to Sex. Total for the Three Years

Month	Male	Female	Total
June	374	1082	1456
July	1267	2299	3566
August	221	429	650
September	283	394	677
October	1	—	1
Total	2148	4204	6350

TABLE X.

Puffers Infested Simultaneously with the Three Major Parasites (Excluding Protozoa).

Size in cm.	No. of Individuals in each class	M	F	I	Copepod	*Lintonium*	*Bianium*
10	4	3	—	—	43	4	15
11	16	5	—	—	27	26	51
12	20	2	4	—	67	34	31
13	18	3	4		59	8	100
14	22	4	—		48	45	42
15	28	6	3		58	42	105
16	32	8	1		66	23	93
17	34	8	5		90	72	261
18	28	7	11		89	135	513
10	32	10	15		205	190	697
20	19	3	9		83	72	424
21	10	0	8		85	33	565
22	8		6		189	30	205
23	8		6		46	34	202
24	1		1		5	1	93
Total	280	61	71	—	1160	749	3397

TABLE XI.

Frequency Distribution of Parasites of Puffers (Excluding Protozoa). In Groups of 25.

Number of Parasites	Male	Female	Immature	Total	Percentage
0	7	7	17	34	10.54
1-25	105	76	3	184	62.65
26-50	25	21		46	15.64
51-75	6	9		15	5.10
76-100	2	8		10	3.43
101-125	0	2		2	.68
126-150	0	0		0	.00
151-175	0	2		2	.68
176-200	0	2		2	.68
201-225	0	1		1	.33
226-250	0	0		0	.00
251-275	0	0		0	.00
276-300	0	1		1	.33
Total	145	129	20	294	100.06

14. It has been shown that the intensity of infection in Sandy Hook puffers reaches a peak in July and sharply drops off in August.
15. It has also been found that the number of parasites increases rapidly with size and age of the fish.
16. Conversely, young and sexually immature puffers are particularly free of parasites.
17. Female puffers are more susceptible than males to the metazoan parasites. Since the heaviest infestation occurred in June and July, it is assumed that this may be attributed to their more intensive feeding preparatory to spawning.
18. There is no evidence to indicate that a heavy infestation of any one species of parasite confers resistance to the host against other species. It has been shown that about 45% of the catch was simultaneously infested with *Pseudochondracanthus*, *Lintonium* and *Bianium* and that this 45% harbored 84% of these parasites.
19. About 63% of the puffers were infested with 1-25 parasites. All puffers harboring more than 100 parasites were females. About 11% of the fish were free of parasites.
20. It is suggested, as a preventative measure, that young puffers (measuring up to 12 cm.) or those collected late in the season (September) be used for exhibition purpose since such fish are comparatively free of detrimental parasites which may spread to other and more important fishes.

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